

AMENDMENTS

IN THE SPECIFICATION:

Please replace the paragraph bridging pages 27-28 by the following paragraph:

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Analogous results were obtained for intravenous introduction of nucleic acids. We tested variations in the quantity of peripheral leukocytes in rabbits 1-3 hours after 100 mg yeast RNA-P or RNA-D solution was injected intravenously. Intravenously injected solution of 0.85% NaCl was used as the standard of non-toxicity. It was demonstrated that, analogously to the standard, an injection of yeast RNA-P or RNA-D does not cause a variation in the number of leukocytes within 3 hours of the introduction. In animals, which took 0.85% solution of NaCl, the quantity of leukocytes was equal to 13000 ± 980 , while those, who had RNA-P or RNA-D, showed accordingly 12700 ± 850 and 12900 ± 980 , which is not abnormal. When the rabbits received injections of 10 mg of proteus polysaccharide, the quantity of leukocytes decreased in 1 hour from 13050 ± 1100 to 2900 ± 210 , and remained at that level while the test lasted (3 hours). These results prove the non-toxicity of yeast RNA. Further, when 100 mg of yeast RNA-P or RNA-D per 1 kg of body weight was given to rabbits intravenously, no acute-phase C-reactive protein was determined, which indicates that there was no endotoxic action.

Please replace the last full paragraph of page 28 by the following paragraph:

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We studied the anti-inflammatory action of nucleic acids on the model of thrombocyte aggregation in vitro by the method of Born (Born L.V.R. The aggregation of blood platelets by diphosphate and its reversal, Nature, V.94, P.327, 1962). Venous human blood was taken in silicon tubes of *Becton Dickson*, which contained a 3.8% solution of sodium citrate. In order to receive thrombocytic-rich plasma, citrate blood was centrifuged at 1500 rev/min for 7 minutes. Plasma free of thrombocytes was obtained by centrifuging 2.0 ml of plasma taken from medium layers for 15 minutes at 3000 rev/min. We counted the number of thrombocytes in the thrombocytic-containing plasma, which was later diluted by the thrombocyte-free plasma to the final concentration $200.0-300.0 \times 10^8/l$.

Please replace Table 2 and the following two paragraphs on page 30 by the following Table and two paragraphs:

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	RNA-D	Aspirin	DNA-CT	DNA-EC	t-RNA
M	59,73	38,66	54,45	36,93	52,23
+ -m	4,24	6,71	3,76	1,88	8,13
		P < 0.02	P < 0.2	P < 0.01	P > 0.5

The test results showed that nucleic acids in the concentration $1 \times 10^{-2}\%$ inhibit aggregation of thrombocytes induced by arachidonic acid. Further, Yeast RNA-D in the concentration $1 \times 10^{-2}\%$ inhibited aggregation of the induced thrombocytes almost twice as effectively as aspirin (38.66%): yeast RNA-D showed 59.73% and transport *E.coli* RNA had 52.23%. DNA from chicken

erythrocytes acted at the same level as aspirin (36.93%), while DNA from cattle thymus inhibited aggregation of thrombocytes by 54.45%, which is almost at the level of yeast RNA. Since DNA always contain a significant amount of RNA, it is probable that the inhibiting effect of DNA can be attributed to the RNA contained in DNA.

Further, an analysis of the influence of different concentrations of yeast RNA on the aggregation of induced thrombocytes showed that yeast RNA was effective in a wide range of concentrations from 0.1% to 1×10^{-5} % and inhibited aggregation by 78.5% and 14.2%, as shown in Table 3 below.

Please replace Table 3 on page 31 by the following Table:

	RNA 0,1%	RNA 1×10^{-2} %	RNA 1×10^{-3} %	RNA 1×10^{-4} %	RNA 1×10^{-5} %
M	78,58	53,08	28,88	43,35	14,23
+ -m	7,51	3,23	1,63	10,3	4,98
		P < 0.01	P < 0.001	P < 0.01	P > 0.001

Please replace Table 4 on page 31 by the following Table:

Conc. 0.1 %	RNA-P	RNA-PN	RNA-F
M	84,09	45,96	57,9
+ -m	3,77	8,96	9,58
		P < 0.001	P < 0.02
Conc. 1×10^{-2} %	RNA-P	RNA-PN	RNA-F
M	71.91	55.44	60.90
+ -m	8,45	8,04	10,39
		P < 0.2	P > 0.5
Conc. 1×10^{-3} %	RNA-P	RNA-PN	RNA-F
M	29,76	3,72	18,26
+ -m	5,36	2,4	5,46
		P < 0.001	P < 0.1

Please replace the last full paragraph on page 32 and the following paragraph bridging pages 32-33 by the following two paragraphs:

In order to evaluate the membrane-stabilizing action of nucleic acids against the influence of free radicals, we calculated the acid resistance of normal rat erythrocytes separated from blood plasma. Rat erythrocytes were rinsed thrice in the cold (4°C) solution of 0.15M of NaCl. The layers of leukocytes and thrombocytes were removed. Acid lysis of the remaining erythrocytes was induced by adding 10 µl of the suspension, which was diluted to the concentration of erythrocytes (0.7×10^6 cells per 1 ml of iso-osmotic medium), and which contained 0.14M of NaCl, 0.01M of the citrate-phosphate buffer pH=2.5, different doses (10 or 100 µg) of nucleic acids, and a stable concentration of nitric sodium, 250 µg per 1 ml, to initiate the oxide damage of erythrocytes.

Erythrocytal lysis was initiated by adding 1 ml 0.004N HCl; changes in existence were recorded at 750 nmol. The method of calculation is explained in Example 6.3. It was demonstrated that yeast RNA-D in the doses of 10 and 100 µg increased the level of total resistance of the

erythrocytes from 288 units (control value recorded for the influence of NaNO_2 without yeast RNA) to 449 units (yeast RNA concentration 10 μg) and 437 units (yeast RNA concentration 100 μg), which is close to norm (475 units). RNA-PN increased total resistance to 328 units in the dose of 10 μg and to 415 units in the dose of 100 μg . RNA-P increased total resistance to 315 units in the dose of 10 μg and to 462 units in the dose of 100 μg (maximally close to the normal level of this indicator). RNA-F increased total resistance to 338 units in the dose of 10 μg and, on the contrary, somewhat decreased (to 271 units) in the dose of 100 μg .


Please replace Table 11 on page 47 by the following Table:

Ischemia 30 min (Control)					Ischemia 30 min + Yeast RNA		
	Norm	Border zone	Infarction zone	Intact	Border zone	Infarction zone	Intact
M	46.500	259.310	185.626	129.655	59.634	115.122	122.630
+ - m	7.000	60.683	48.635	30.341	11.649	40.509	26.413
P1		<0.01	<0.05	<0.2	<0.5	<0.1	>0.05
P2					<0.2	<0.5	<0.05

Please replace Table 13 on page 49 by the following Table:

Ischemia 30 min (Control)					Ischemia 30 min + Yeast RNA		
	Norm	Border zone	Infarction zone	Intact	Border zone	Infarction zone	Intact
M	4.827	9.910	9.716	9.813	7.270	8.530	7.900
+ - m	0.378	1.003	0.947	0.919	0.456	0.741	0.493
P1		<0.01	<0.01	<0.001	<0.01	<0.01	<0.01
P2					>0.05	>0.05	>0.5

Please replace the last two full paragraphs of page 53 and the following paragraph bridging pages 53-54 by the following three paragraphs:



As shown in Table 15, the control group of animals showed a substantial increase of NOS activity on the 3rd and 14th day of auto-immune pathology in comparison with norm (30.65 ± 7.35 picomol per 1 min per 1 mg of protein in norm, 236.76 ± 76.42 picomol per 1 min per 1 mg of protein on the 3rd day, and 111.54 ± 15.78 picomol per 1 min per 1 mg of protein on the 14th day). Such a significant increase in the activity of NOS indicates that activity of the inducible NOS-isoform (iNOS), whose synthesis is initiated by anti-inflammatory cytokines INF- γ , IL-1 β , TNF- α , et. al., is the main compound in the calculated activity of NOS.

In the period between the 3rd (initiation of the auto-immune process) and 14th day (development of pathology), we observed a normalization in the activity of NOS in blood (24.34 ± 8.60 pmol per 1 min per 1 mg of protein). This may probably be attributed to the activated protective reaction of body, and could be induced by inhibition of the expression of NOS as well as by modulation of the stability of its mRNA, or by inhibiting the process of its translation.

In the group of animals which took yeast RNA, initiation of the auto-immune process (on the 3rd day) was accompanied by a much smaller (in comparison with the control group) increase in the activity of NOS in blood (70.00 ± 9.24 pmol per 1 min per 1 mg of protein against 236.76 ± 76.42 pmol per 1 min per 1 mg of protein). Moreover, the activity of NOS decreased progressively over the next

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period in development of auto-immune process (40.66 ± 5.05 pmol per 1 min per 1 mg of protein on the 8th day and 33.96 ± 6.04 pmol per 1 min per 1 mg of protein on the 14th day).

Please replace the paragraph bridging pages 54-55 by the following paragraph:

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The level of damage in erythrocytes under the influence of various harmful factors in the course of an auto-immune process was evaluated by kinetic indicators of hemolysis, induced by a pH decrease in the environment. Kinetic indicators of hemolysis were recorded; the number of damaged cells was determined spectrophotometrically in equal periods of time (30 s) by changes in the value of integral light dispersion of erythrocytal suspension ($\lambda=750$ nmol). Absorption spectra were registered by a spectrometer SF-26 (Russia). Acid lysis of erythrocytes was initiated by adding 10 μ l of blood, which was diluted 20 times in the isotonic medium 0.14 mol of NaCl + 0.01 mol of the citrate-phosphate buffer with pH=2.0-3.5 (volume: 1 ml; density of erythrocytes in suspension: 0.7×10^6 cells per ml). For such densities, the value of integral light dispersion of erythrocytes depends on the count, size, and shape of cells and is proportional to the number of cells in suspension.

IN THE CLAIMS:

Please amend claims 1-5 and 20 as follows:

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1. (Amended) A method for the prevention or treatment of inflammation or inflammatory-related disorder comprising administering to a mammal in need of such treatment a composition comprising total yeast ribonucleic acid and a pharmaceutically acceptable vehicle, carrier, or diluent,

said composition comprising said ribonucleic acid in an amount ~~effective to ameliorate symptoms of~~
inflammation or inflammatory-related disorder, wherein said composition is administered so that said
ribonucleic acid is present into the mammal's blood.

2. (Amended) A method of stabilizing acid-challenged erythrocyte membranes of a mammal
in need of prevention or treatment of inflammation or an inflammatory-related disorder, which
comprises administering to said mammal a composition comprising total yeast ribonucleic acid and
a pharmaceutically acceptable vehicle, carrier, or diluent, said composition containing said total yeast
ribonucleic acid in an amount effective to stabilize said acid-challenged erythrocyte membranes,
wherein said composition is administered so that said ribonucleic acid is present into the mammal's
blood.

3. (Amended) A method of inhibiting oxidation into arachidonic acid of components of cell
membranes of a mammal in need of prevention or treatment of inflammation or an inflammatory-
related disorder, which comprises administering to said mammal a composition comprising total yeast
ribonucleic acid and a pharmaceutically acceptable vehicle, carrier, or diluent, said composition
containing said total yeast ribonucleic acid in an amount effective to inhibit oxidation into arachidonic
acid of components of cell membranes of the mammal, wherein said composition is administered so
that said ribonucleic acid is present into the mammal's blood.

4. (Amended) A method of reducing an amplitude of variations of NO-synthetase activity
induced by inflammation or inflammatory-related disorder in a mammal, which comprises
administering to said mammal a composition comprising total yeast ribonucleic acid and a
pharmaceutically acceptable vehicle, carrier, or diluent, said composition containing said total yeast

ribonucleic acid in an amount effective to reduce the amplitude of the variations of NO-synthetase activity in the mammal, wherein said composition is administered so that said ribonucleic acid is present into the mammal's blood.

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5. (Amended) A method of inhibiting thrombocyte aggregation induced by inflammation or inflammatory-related disorder, which comprises administering to a mammal in need of such treatment a composition comprising total yeast ribonucleic acid and a pharmaceutically acceptable vehicle, carrier, or diluent, said composition containing said total yeast ribonucleic acid in an amount effective to inhibit thrombocyte aggregation, wherein said composition is administered so that said ribonucleic acid is present into the mammal's blood.

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~~20. (Amended) A pharmaceutical composition for the treatment or the prevention of inflammation or inflammatory-related disorder, comprising total yeast ribonucleic acid and a pharmaceutically acceptable vehicle, carrier, or diluent.~~

Please add new claims 23-38 as follows:

23. (New) A method in accordance with claim 1, wherein said composition is administered by interabdominal injection.

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24. (New) A method in accordance with claim 1, wherein the inflammation or inflammatory-related disorder is an acute inflammatory event involving short term effects.

25. (New) A method in accordance with claim 2, wherein the inflammation or inflammatory-related disorder is inflammatory swelling.

26. (New) A method in accordance with claim 25, wherein the inflammatory swelling is carageenan-induced.

27. (New) A method in accordance with claim 2, wherein the inflammation or inflammatory-related disorder is auto-immune inflammation.

28 (New) A method in accordance with claim 27, wherein the auto-immune inflammation is adjuvant arthritis.

29. (New) A method in accordance with claim 2, wherein the mammal is a rat or a mouse.

30. (New) A method in accordance with claim 3, wherein the mammal is a rat or a mouse.

31. (New) A method in accordance with claim 4, wherein the mammal is a rat or a mouse.

32. (New) A method in accordance with claim 5, wherein the mammal is a rat or a mouse.

33. (New) A method in accordance with claim 1, wherein said composition is administered so that ribonucleic acid is present into the mammal's blood prior to occurrence of the inflammation or inflammatory-related disorder.

34. (New) A method in accordance with claim 2, wherein said composition is administered so that ribonucleic acid is present into the mammal's blood prior to occurrence of the inflammation or inflammatory-related disorder.

35. (New) A method in accordance with claim 3, wherein said composition is administered so that ribonucleic acid is present into the mammal's blood prior to occurrence of the oxidation into arachidonic acid of components of cell membranes.

36. (New) A method in accordance with claim 4, wherein said composition is administered so that ribonucleic acid is present into the mammal's blood prior to occurrence of increased NO-synthetase activity.

37. (New) A method in accordance with claim 5, wherein said composition is administered so that ribonucleic acid is present into the mammal's blood prior to the inflammation or inflammatory-related disorder.

38. (New) A pharmaceutical composition in accordance with claim 20, wherein nucleic acids contained in the composition consist essentially of said total yeast ribonucleic acid.